

## Invited Review

# Leprosy as a Challenge to Science on the Ability to Decode Its Enigma. A Hypothesis on How to Respond

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### CONTENTS:

1. Introduction
2. Incomplete current etiology of leprosy
3. Modern Japanese pathologists' view on the etiology of diseases and its application to leprosy by Mitsuda leading to the departure from Koch's postulate
4. An example of human disease showing dual infection and those of syntrophism in nature
5. An overview of the rejected and neglected evidence of dual infection in leprosy, including those of the author's, which might contain the latchkey to open the riddle
6. On the *HI-75* after the denials to be cultured *M. leprae* and identified as *M. scrofulaceum*
7. The discussion on how to decode the enigma of leprosy by the study of *HI-75* or the other *M. X*

**SUMMARY:** In world leprosy nowadays, a favorable epidemiologic trend has been provided due to the best effort of the worldwide campaign with chemotherapy providing a bright but one-sided look at the future. However, the numbers of new patients are still higher than those under chemotherapy, leaving a concern over the remaining non-human source of infection. To overcome that plausibility, overall understanding of the etiology of the disease should be improved. The author discussed this by the analyses of historical and scientific legitimacy of the current idea about the etiology of leprosy that have unreasonably rejected the possibility of dual infections in relation to that of *Mycobacterium leprae*. The analyses also consider the author's ongoing effort to know the feasibility of artificial culture of *M. leprae* by improving of the former methods reported by Skinsnes et al. and has been rejected as it contained *Mycobacterium scrofulaceum*, without attention to the coexistent *M. leprae* at that time. The bacillus thus maintained with the modification of the medium still shows PGL-1 immunoreactivity and the pathogenicity to cause neuropathy in mice. These strongly suggest the coexistence of the above two bacilli throughout past years. The genomic study is in progress to prove that hypothesis, the genomes should be alike in nature if proven.

### 1. Introduction

At the beginning of 2004, the number of leprosy patients under treatment in the world was around 460,000 (available from: <http://www.who.int/lep/>). About 515,000 new cases were still detected during 2003. Among them, 43% were multibacillary cases, 12% were children, and 3% had severe disabilities already. During the past 2 years, the global number of new cases showed a per year reduction of 20% according to the World Health organization (1; <http://www.who.int/lep/disease.htm>). These numbers are generally taken as a miracle comparing with those before the age of multidrug therapy when the exact numbers of patients was too difficult and large to gather from every area of the world. However, that trend of epidemiology has been established only after the best effort of worldwide campaign with chemotherapy (1).

As the epidemiology shows, there are still two remaining concerns. The one is the larger number of new patients per year than those under chemotherapy. In this respect let us not disregard Meyers et al.'s (2), Lechat's (3) likewise Chakrabarty and Dastidar's (4) reports alerting us to the possibility of infection sources besides patients. The other major concern

is about disabilities which are a result of this disease, and exact total worldwide numbers of which are not listed in these reports, and therefore remained only a guess. For some who believe human-to-human contagion is the only source of leprosy, the solution for the above two concerns might be obtained by decades' long quality control of the record of each new patient to rule out those repeating chemotherapy.

To cope with the situations, a better overall understanding of the disease is necessary and might be possible if we take the matter more substantially by looking at the history of fundamental research, for an understanding of the present epidemiology and countermeasures in the future. That is the reason why the author attempts to understand the imperfect knowledge about the scientific basis of leprosy and the enigma therein, especially concerning the etiology itself, challenging the ability of scientists how to decode it.

### 2. Incomplete current etiology of leprosy

Historically, the discrimination of devilry and the divine as the causes of diseases in medicine was made by Hippocrates of Cos and his schools back in 5 to 4 BC in Greek. That has made him the patriarch of pathology. In later history, especially after the Renaissance, the philosophy of how to view the causes of diseases became to be much more substantial and has now developed to seek the etiology of diseases. The medical sciences, among them especially pathology in modern

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times, originated from that basis (5).

In leprosy, the most important etiologic agent, *Mycobacterium leprae* was discovered by Gerhard A. Hansen in 1873. That was contemporary with the establishment of cellular pathology by Rudolph Virchow and his disciples (5) who also contributed to an understanding of the pathology of leprosy. From that time on, the discoveries of etiologic microorganisms have been followed by the enough understanding to take practical countermeasures for many diseases. This was not, however, the case for leprosy. Luckily the development of chemotherapy in the later half of the 20th century outpaced the fundamental understanding of leprosy.

In microbiology, classical and molecular, Koch's postulates have been applied to identify etiologic microorganisms for infections (6). The application of these postulates usually have led the search to a single microorganism as the cause of a disease. However, in leprosy the postulate has never been fulfilled since *M. leprae* is often not recovered from patients of its tubercloid type and the bacillus have not been thought to have been artificially cultured (7). Concerning the failure of cultivation of *M. leprae* over the past 130 years, the gene decay of *M. leprae* as reported by Cole et al. (8) may help to explain this difficulty. However, the concept does not correspond with the reports of Kazda et al. (9) who confirmed the acid fast mycobacterium with *M. leprae* specific immunoreactivity in the compressed vegetation of sphagnum moss and Mostafa et al. (10) that genome could be found in the soil of Norway long after the leprosy patients all died. It is also hard to understand that either the non-proliferating bacillus or its genome in the soil could withstand the natural decomposition or dilution for many decades. That skepticism might coincide with the proposition by Chakrabarty and Dastidar (4) that leprosy from non-human or environmental sources was built up to man and animals. These, rather, support the possibility that *M. leprae* can grow in nature as Hanks (11) once suggested, i.e., that the lack of growth competence in vitro does not prevent survival in nature. This is also consistent with the concern as raised by Meyers et al. (2), Lechat (3) as well as Chakrabarty and Dastidar (4) that naturally growing *M. leprae* also become sources of infection, in addition to human patients or animals. The solution to the enigma of how the genome of *M. leprae*, in other words, *M. leprae* itself, could survive with the moss or in soil, can be reached by knowing the growth conditions of *M. leprae* in culture or vice versa, which will help to understand the countermeasures to be followed. Though Hanks expressed his view to the investigators favoring the cultivation with the reproving words 'why bother', introducing his conviction that genetic engineers are totally antagonistic to cultures (11). However, the researcher's advantage is usually not because of their obstinacy but of their objectivism.

### 3. Modern Japanese pathologists' view on the etiology of diseases and its application to leprosy by Mitsuda leading to the departure from Koch's postulate

Ogata and Ohta once wrote in their textbook "An introduction to pathology", as follows: more often than not if we pry into the etiology of a certain disorder, we recognize that the disease was caused by many etiologic agents. However hard we pursue those, it is almost impossible to know all of them. Therefore, in pathology, we name the most principal inciting cause of the affliction as the main reason, which is supported by accessory or exciting causes. That means that

they had the concept that most sicknesses, with only quite limited exceptions, are created by many etiologic agents (12). However, they did not express the extension of their view to principal and accessory microbiotic agents to cause an infectious disease themselves. However, the answer can be speculated from two documents which were written by Mitsuda (13) who once was affiliated with the same department of pathology, Tokyo University School of Medicine, as that of Ogata and Ohta. Mitsuda and Ogata lived contemporaneously.

The first report was a biopsy report by Mitsuda in 1902 about a cervical lymph node of 58-year-old patient, suffering from both lepromatous leprosy and tuberculosis of right lung. Mitsuda concluded in this report as follows: in lymph node, *Mycobacterium tuberculosis* and *M. leprae* often make tuberculous and leprosy lesions. In such case, *M. leprae* invades into tuberculous lesions and multiply along the lines of the nuclei of Langhans' giant cells and form specific globi. In addition, *M. leprae* also parasitizes in epithelioid cells and caseous lesions of tuberculosis. In this respect, Hansen's opinion condemning the lesions with giant cells or caseous lesions was caused only by tuberculosis seems to be extremely discriminative (13). The second report is a microscopic description handwritten by Mitsuda 50 years later which shows the transformation of a granuloma caused by tuberculosis in an inguinal lymph node, to a leprosy lesion (14) (Fig. 1), which is the close-up of the original which was shown by the inset. Note acid fast bacilli in the epithelioid and Langhans' giant cells which rarely occur in case of tuberculosis, therefore he concluded that it was *M. leprae*. The term tubercle bacilli at that time contained *Mycobacteria tuberculosis, intracellulare, bovis*, etc. What he wanted to show by the figure is consonant with that of his report in 1902 which can not be easily denied by pathologists even nowadays.

Therefore, it is almost certain that Mitsuda might have supported the idea of a dual infection besides that of *M. leprae* in leprosy. If the author's speculation is correct, Mitsuda should be the first one who proposed the possibility of dual infection including an organism, in leprosy besides *M. leprae*, which is a theoretical departure from Koch's postulate to explain the etiology of leprosy. The unusually high rate of the coexistence of tuberculosis in leprosy patients had also been reported repeatedly in the past (15) though the histologic descriptions of those did not go into detail as those of Mitsuda (13,14).

### 4. An example of human disease showing dual infection and those of syntrophism in nature

According to the textbook of microbiology edited by Prescott et al. (6), hepatitis D is an example of dual infections. Hepatitis D virus (HDV) is dependent on the hepatitis B virus to provide the envelope protein (HBsAg) for its RNA genome. Thus HDV only replicates in liver cells in which hepatitis B virus also is actively replicating (6). In nature, the condition in which the growth of an organism depends on another organism is designated as syntrophism. The case of mutualism in which both organisms benefit is known as cross-feeding or satellite phenomenon (6). A very important syntrophism occurs in anaerobic methanogenic ecosystem such as sludge digesters, anaerobic freshwater aquatic sediment, and flooded soils (6). Such a dual infection and syntrophisms might be applicable to understand some aspects of leprosy and *M. leprae* which are as written previously.

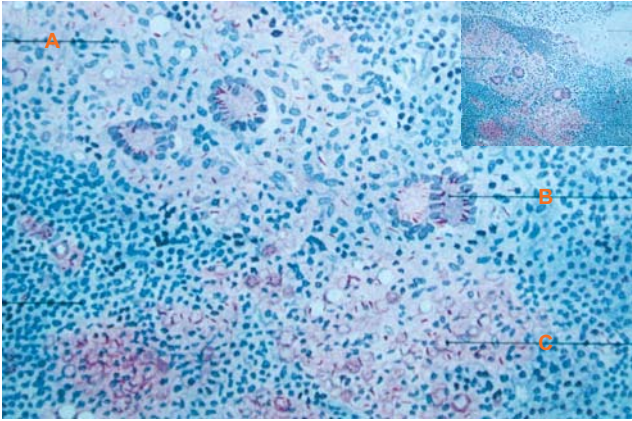


Fig. 1. Mixed leprosy and tuberculous infection in an inguinal lymph node. Ziel-hematoxylin stain (Mitsuda, K.).  
A: Mixture of tubercle and lepra bacilli; B: *Lepra bacilli* within Langhans' giant cell; C: Lipoid degeneration of lepra cells.  
(The authorization for the introduction of this figure was made by the courtesy of Dr. Masanao Makino, Director-General, National Sanatorium Oku-Komyo-En in charge of this matter.)

### 5. An overview of the rejected and neglected evidence of dual infection in leprosy, including those of the author's, which might contain the latchkey to open the riddle

It is certain that the infection of *M. leprae* alone, without dual infection is present similar to the observation that nude mice develop experimental leprosy lesions by the inoculation of *M. leprae*. That unitary infection concept is the dominant concept to explain the etiology of every case of leprosy, leaving many unanswered questions neglected and unsolved. The introduction of a dual infection hypothesis in early stage of leprosy does not interfere with the importance of unitary infection of *M. leprae* in patient once the infection was established. The reason is simple, since the macrophages of lepromatous leprosy patients have the ability to digest *M. tuberculosis*, etc. but not *M. leprae* (16). This observation is consonant with that of Mitsuda (13). That interpretation may not be easily acceptable to current immunologists, however, since the accumulation of host enzymes by enzyme binding proteins (BGBP) (17,28) may represent the way *M. leprae* sneak through the immune defense system with hyaluronic acid (HA) as the shield as well as the nutrient. This is supported by the evidence from the histochemistry of leprosy lesion (17) and the culture results as described later (Figs. 2-4 and 6). Anyway, if we apply a dual infection concept, it is much easier to understand the following experiences of researchers including those of the authors'.

*Mycobacterium X* is a designation that has been applied to *mycobacteria* which have been recovered from leprosy patients and cultivated thereafter in various culture media, after Kato (18) applied the name first time to his bacillus. He identified one of his isolate as *Mycobacterium avium-intracellulare* (19) and suggested the mixture of *M. leprae* in its early subcultures. Concerning about the *Mycobacterium scrofulaceum* which should be classified into essentially same category as that of *M. X* except lepromata were derived from an armadillo, previously infected with *M. leprae* were also reported separately by Matsuo et al. (26) and Nakamura et al. (27).

Also the experience which could be classified into the same category of *M. X* is that of Skinsnes et al. including the present

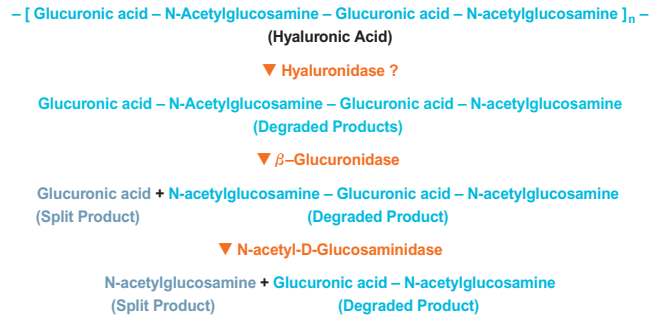


Fig. 2. Hyaluronic acid degradation by enzymes in leprosy lesion.

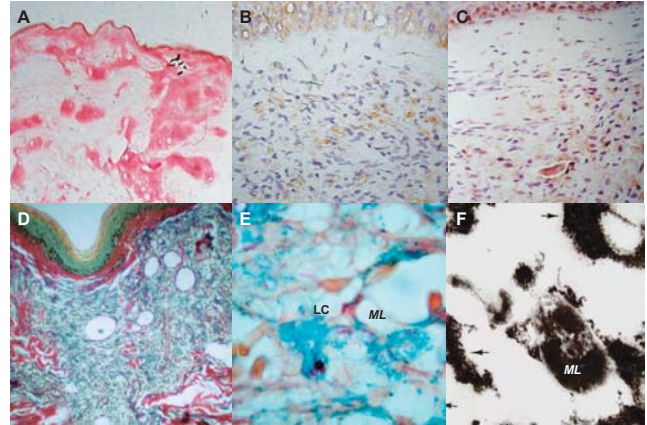


Fig. 3. Hyaluronic acid, degradation by enzyme, its mechanism and *M. leprae* in cutaneous leproma.  
A: Histochemical stain for β-glucuronidase (red) in leproma. 40×; B: Immunoperoxidase stain for human β-glucuronidase (brown). 200×; C: Immunoperoxidase stain for β-glucuronidase binding protein (brown). 200×; D: Hyaluronic acid (blue) in leproma. 100×; E: Coexistent *M. leprae* (ML) and hyaluronic acid (blue) in lepra cell (LC). 400×; F: Electron microscopy; Hyaluronic acid (arrows) around *M. leprae* (ML). 20,000×.

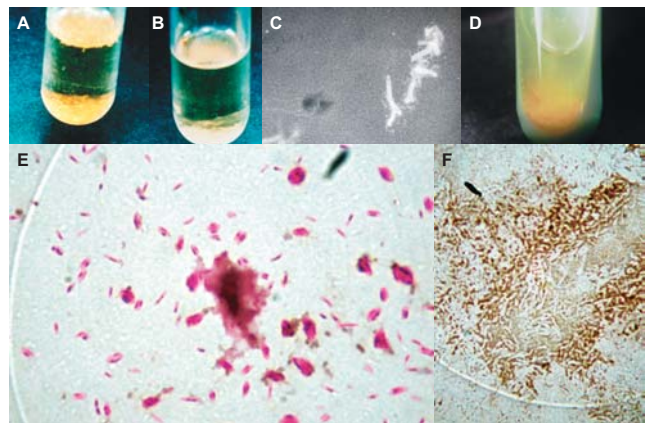


Fig. 4. Culture tubes of HI-75 with the reflection of the histochemistry of leprosy lesion enhancing the immunologic stain for *M. leprae*.  
A: *M. scrofulaceum*; B: HI-75 in 1975; C: Immuofluorescent microscopy for HI-75 in 1975 (modified Abe's method for *M. leprae*); D: HI-75 in 2004; E: HI-75 in 2004. Immunoperoxidase stain for PGL-1 (brown) with Acid fast stain (red). 1000×. Note PGL-1 positive and negative bacilli; F: HI-75 in 2004. Immunoperoxidase stain for PGL-1 (brown). 1000×. Note PGL-1 positive and negative bacilli.

author who claimed the cultured bacillus to be *M. leprae* and named it HI-75 in 1975 (20). The claim was followed by rejections for many reasons. These included the lack of resem-

blance of the mode of its growth to known *Mycobacteria*, the lack of the resemblance of fingerprinting pattern to that of *M. leprae*, lack of *M. leprae* genome, or the growth ability itself in the artificial culture media. However, most of these reasons for rejection never appeared in the publications. Among those published, Stanford et al. (21) noted the resemblance of the claimed bacilli to the characteristics of *M. scrofulaceum*. The author has understood this report to mean that a fairly large proportion of *M. scrofulaceum* besides *M. leprae* had been grown in the cultured bacilli from the start to many years thereafter since the genome of *M. scrofulaceum* was shown to be present in *HI-75* by Sakai et al. including the present author even in 1999 (22). The difference of *HI-75* in 1975 (Fig. 4B) from *M. scrofulaceum* was the whitish haze of the culture fluid superimposing the orange yellow one, characteristics of *M. scrofulaceum* (Fig. 4A), which is essentially same in 2004 (Fig. 4D). The immunological identification of *M. leprae* in the bacillary smears were both positive as shown by Figs. 4C likewise 4E and F. Though, the methods used for the immunological identification of *M. leprae* in 1975 (23, 24), (Fig. 4C) and 2004 (25) (Figs. 4E, F.) were different each other, those were the most advanced and accurate methods at that time. Therefore, the accurate name of *HI-75* could be *complexus mycobacteriae leprosum et scrofulaceum HI-75* if it contained *M. leprae*. Contrarily these types of the studies have been taken as the additional evidence of unsuccessful culture of *M. leprae*, regarding Koch's postulate as the only applicable posit or mantra for the artificial culture of *M. leprae*, without attention to the possibility of mixtures of *M. leprae* within them. The method to identify *M. leprae* by immunological means were already available from 1975 on (23-25) though there are some reports denying specific reactivity to *M. leprae* which will be written later. Even so, the author thinks it better to use such methods, and take the hypothesis of dual infections if positive and follow inductive inference and logic which is generally applicable in the field of medical science, avoiding syllogism to draw conclusions. If so, the presence of *M. X* besides *M. leprae* in leprous lesions should be taken as evidence of success and not as failures of researchers who are skilled in the procedures taken.

## 6. On the *HI-75* after the denials to be cultured *M. leprae* and identified as *M. scrofulaceum*

The composition of the culture medium for *HI-75* has been maintained reflecting the chemical environment similar to that of leprous lesion from the start, and adjusted according to additional information, taking the presence of *M. scrofulaceum* as evidence of success. The main component of culture medium, HA was replaced by its split products, namely glucuronic acid (GA) and N-acetylglucosamine (NAG) (Fig. 2) (28). The reason was that *HI-75* can not obtain GA and NAG by enzymatically splitting HA in culture tube since the bacillus can not produce the enzyme which is able to work so (28). As the basis of that reason, histochemical and immunohistochemical studies showed that *HI-75* in leproma produce a kind of lectin, which is BGBP (28) and accumulate  $\beta$ -glucuronidase (B-Gase) from host human (Fig. 3B) to hydrolyze HA (Fig. 2). Thus the bacillus can get GA and NAG from host human HA. Therefore, the component of the culture medium for *HI-75* should be GA and NAG but not HA. N-acetylglucosaminidase (NA-Gase) should work as B-Gase since that takes a similar location in

leprous lesions (17) and has the ability to combine with lectins (29). Although, the author has not confirmed the participation of hyaluronidase, that may not be important since both B-Gase and NA-Gase can split GA and NAG in the tail ends of HA (Fig. 2). Our failure to report the detail of immunohistology showing human B-Gase in leproma is the result of procedures in tissue processing which hamper staining in some cases. The antibody preparation against human B-Gase and its application to the cases of nephropathy were reported elsewhere (30,31). The change from the original to Ogawa's medium (3%) to culture *HI-75* was due to the commercial availability of Ogawa's medium which makes the rest of the modification much simpler (28).

In addition, since cohabitation of *M. leprae* and *M. scrofulaceum* in *HI-75* has been highly suggested (20,23,24) (Figs. 4C and 4E-F), the nerve lesions due to *HI-75* inocula-

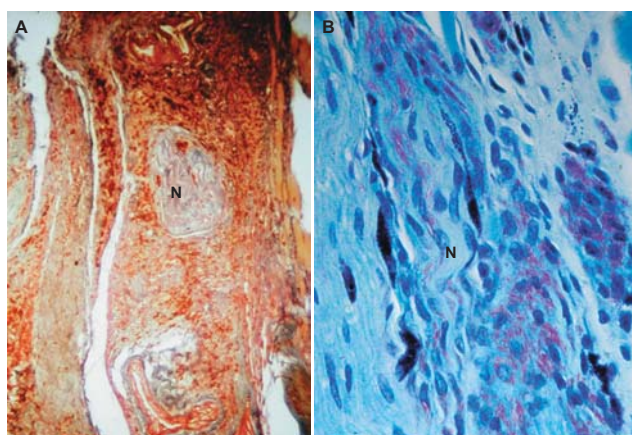


Fig. 5. Nerve invasion (N) and growth of *HI-75* (red). A: Nude mouse footpad a year after the injection of 0.5 million *HI-75*. Harada's Acid-Fast Stain. 100 $\times$ . (The photomicrograph was provided for the publication by the courtesy of Dr. Norisuke Sasaki.) B: Nude mouse cheek. 4 months after injection of 11 million *HI-75*. Nyka and O'Neill's Acid-Fast Stain. 400 $\times$ . (The original mouse tissue for the processing and publication was made by the courtesy of Dr. Hamit, Sidik after his publication.)

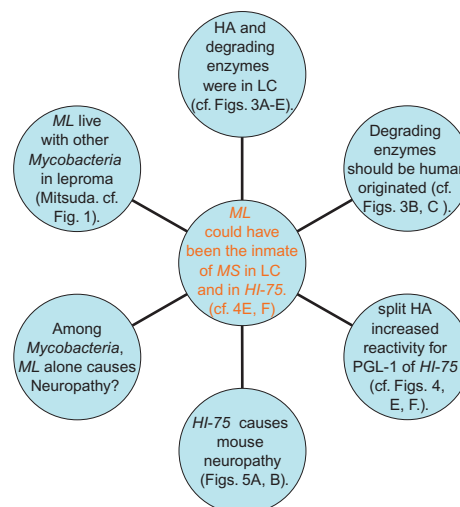


Fig. 6. The inductive inference and logic leading to the conclusion in center and further heading for the possible elucidation of nature-parasites (*ML*, *MS*)- host-relationship in leprosy. HA: Hyaluronic acid; LC: Leptra cell; *ML*: *M. leprae*; *MS*: *M. scrofulaceum*.

tion into experimental animals should reinforce the hypothesis of dual infection in leprosy. On the ability of *HI-75* to cause neuropathy of mice, Stanford et al. (21) and Kamala et al. (32) denied that. However, Sasaki et al. (33), Hamit (34) and Furuno (35) reported that ability. Fig. 5A shows a photomicrograph of a mouse footpad with the growth of numerous acid fast *mycobacterium*, namely *HI-75*, which stained red, in a nerve (N) and the tissue encompassing that. Fig. 5B shows several bundles of peripheral N of a nude mouse. Note especially a N which shows the apparent intraneural growth of many acid fast *mycobacterium* which stained red. This means that if *HI-75* does not contain *M. leprae*, the whole concept of identification of *M. leprae* could collapse since the nerve invasion itself has been regarded as a pathognomonic finding for leprosy (7) as illustrated in Fig. 6.

Therefore, again, if the standpoint of inductive inference and logic are taken, *HI-75* seems to have the key to decode the enigma of leprosy especially concerning *M. leprae*. The effort to firm up the ground further by finding the genome itself of *M. leprae* in *HI-75* is still going on and will be reported in future.

#### 7. The discussion on how to decode the enigma of leprosy by the study of *HI-75* or the other *M. X*

As written before, the presence of *M. leprae* in nature such as in the vegetation of sphagnum moss or in the soil of Norway can not be denied. The dual infection of an organism besides *M. leprae* in leprosy also may not be denied either as described previously. In such a condition artificial culture of *M. leprae* if possible could be the first step to understand the environment how *M. leprae* survives in nature and patients or vice versa. The author's attempt utilizing *HI-75* has shown the growth of most likely two bacilli in the same culture tube as described previously. The one seems to be *M. scrofulaceum* and the others was not identified so far by genomic study but it could be *M. leprae* since *HI-75* grew in nerves of mice and caused neuritis. The bacillus can be immunologically stained utilizing modified FLA-ABS (24) or anti-PGL-1 antibody. The next step, therefore, should be the extensive genomic study to look for the mixture of *M. leprae* in *HI-75*. The study should be the simulation of the conditions in which the genome of *M. leprae* has been detected from patients, animals or in nature removing inhibitors of the test considering the difference which might be in handling the cultured bacillus.

In case extensive genomic studies can not confirm the mixture of *M. leprae* in *HI-75* or other examples of *M. X* with the capability to cause neuritis, that ability should be removed from the rank of pathognomonic lesion caused by *M. leprae*. Even so, the experimental peripheral neuritis and immunological tests are helpful to corner *M. leprae* into culture tubes for further study even if the arguments to deny the specificity of PGL-1 to *M. leprae* exist (36,37). The reason is that even if individual test may not be absolutely reliable, cumulative results can often leads to a correct answer. That is the way of inductive inference and logic to avoid fatal mistake otherwise and therefore regarded as an unparalleled science of reasoning in medicine.

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